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METABOLIC ENCEPHALOPATHIES

The role of ammonia, amino acids and
blood-brain barrier derangement



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METABOLIC ENCEPHALOPATHIES

The Role of Ammonia, Amino Acids and
Blood-Brain Barrier Derangement

by

Bengt Jeppsson

Lund 1981

We dance round in a ring and suppose,
But the Secret sits in the middle and knows.

Robert Frost, "The Secret Sits".



This thesis is based on the following papers:

- I Hyperammonemia, plasma aminoacid imbalance, and blood-brain aminoacid transport: a unified theory of portal-systemic encephalopathy. James, J.H., Ziparo, V., Jeppsson, B. and Fischer, J.E. Lancet ii: 772-776, 1979.
- II The role of brain glutamine in blood-brain neutral amino acid transport after portacaval anastomosis. James, J.H., Jeppsson, B., Edwards, L., Brenner, W. and Fischer, J.E. Submitted for publication.
- III Effects of administration of ammonium-salts on plasma and brain neutral amino acids. Jeppsson, B., Ziparo, V., James, J.H., Brenner, W. and Fischer, J.E. Submitted for publication.
- IV Increased blood-brain transport of neutral amino acids after portacaval anastomoses in germ-free rats. Jeppsson, B., James, J.H., Hummel, R.P., Brenner, W., West, R. and Fischer, J.E. Submitted for publication.
- V Blood-brain barrier derangement in sepsis: cause of septic encephalopathy? Jeppsson, B., Freund, H.R., Gimmon, Z., James, J.H., von Meyenfeldt, M.F. and Fischer, J.E. Am. J. Surg. 141: 136-142, 1981.
- VI Blood-brain barrier derangement in uremic encephalopathy. Jeppsson, B., Freund, H.R. Gimmon, Z., James, J.H., von Meyenfeldt, M.F. and Fischer, J.E. Submitted for publication.

The above articles are referred to in the text by the Roman numerals.

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METABOLIC ENCEPHALOPATHIES - CURRENT CONCEPTS ON PATHOPHYSIOLOGY

Patients with liver failure or portal-systemic shunting of blood, renal failure, and sepsis often manifest symptoms of cerebral dysfunction. The symptoms are referred to as metabolic encephalopathies and include disturbance of mental state and neuromuscular abnormalities. The patient may exhibit alterations in judgement, personality, and mood (either euphoria or depression). There may be inappropriate behavior, and sometimes there is inversion of sleep pattern, or the patient may be drowsy or asleep for long periods. If the encephalopathy progresses, the patient becomes more confused and difficult to arouse and finally passes into coma.

Asterixis or flapping tremor is probably the most characteristic sign of neuromuscular abnormality in metabolic encephalopathies. Other signs are usually transient and include muscular rigidity and hyperreflexia.

Underlying pathophysiological mechanisms have not been clearly identified, although recently the knowledge has increased considerably.

Portal-systemic encephalopathy

The association between the injured liver and/or portal-systemic shunting and impaired mental state has long been known and has given rise to a variety of general terms. Better understanding of the pathogenesis has led to better, more descriptive terms and the most precise functional terminology now in use is portal-systemic encephalopathy (PSE) (1).

PSE seems to be a metabolic/neurophysiologic disorder in most instances, in that the disorder is potentially fully reversible without residual sequelae and important structural lesions in the brain. PSE also appears to be a multifactorial problem. Several metabolic abnormalities have been incriminated as specific causes of PSE. None of these are pathognomonic for the development of the disease in all instances, but they must be evaluated in understanding the pathogenesis of PSE.

Ammonia

Disturbed ammonia metabolism is in some way basic to the syndrome of PSE. Ammonia first came to the fore with the recognition that the meat intoxication which affected animals with Eck fistula could be mimicked by the oral administration of ammonia (2).

Increased blood ammonia is found in many patients with PSE, and blood ammonia determination remains the most commonly used laboratory test in the diagnosis of PSE.

An involvement of ammonia in the pathogenesis of PSE is suggested by a positive correlation between blood ammonia levels and the presence and degree of PSE. Many authors have reported a close posi-

tive correlation (3,4); others have shown a poor correlation (5). In my opinion, the correlation is good, but not perfect. Another indication of a role of ammonia in PSE is the reported beneficial effects of lowering plasma ammonia by antibiotics and lactulose or reduction of protein intake on PSE (6,7).

Ammonia generation. The gastrointestinal tract, kidneys, and skeletal muscle are the major sources of ammonia. The gut was identified as the major source of ammonia by the work of Hahn (2). He described intermittent stupor in Eck fistula dogs after protein feeding, and was able to reproduce it by administration of ammonium chloride.

Folin and Denis confirmed in cats that the portal venous blood ammonia rose after a meal (8). These observations, combined with clinical evidence that a high protein diet in susceptible patients with liver disease caused a rise in blood ammonia, suggested that unabsorbed protein was deaminated by bacteria in the colon from where ammonia was absorbed, and also accounted for the fall in blood ammonia in a patient given a low protein diet, cathartics, and oral antibiotics (6,7). Bacterial hydrolysis of urea is another gastrointestinal source of ammonia (9).

Recently it was observed that germ-free animals that have undergone a portacaval anastomosis develop meat intoxication with a commensurate rise in blood ammonia (10). Thus sources of ammonia other than bacterial degradation of protein or urea must also be involved. Weber and Veach have shown in normal dogs that small bowel contributes as much ammonia as large bowel (11). The amount of ammonia released by the small intestine was directly correlated with the amount of glutamine taken up from arterial blood, indicating endogenous glutamine as the main substrate for intestinal ammonia production.

The kidney is important in ammonia metabolism. Not only is it the organ which actually excretes the terminal, nitrogenous residues from the body, but it also generates ammonia. Probably its most important ammonia-generating reaction is the deamidation of glutamine (12).

Ammonia is also released from exercising muscle (13).

The highest blood ammonia levels in the body are found in the portal vein. During its initial passage through the liver, 70 - 80 percent of the ammonia is removed (14). The hyperammonemia of liver failure and portal-systemic shunting represents both a decreased capacity to process ammonia and shunting of ammonia rich portal blood around the liver.

Ammonia toxicity. The mechanism by which ammonia exerts its deleterious effects is obscure. Indeed, the large number of mechanisms postulated attests to this (15). The most widely postulated mechanism of action is its interference with brain energy metabolism. The concept that ammonia suppresses cerebral energy metabolism is based primarily on the effects of the biochemical detoxification of ammonia in the brain. Ammonia is removed by the synthesis of gluta-

mine from glutamic acid. This pathway of removal may result in depletion of α -ketoglutarate, which, in turn, may interfere with the orderly operation of the tricarboxylic acid cycle (16). This reaction may also decrease the availability of ATP by excessive utilization of ATP, which is the obligatory energy source of the glutamate-glutamine reaction. This explanation is no longer tenable for several reasons. Depletion of α -ketoglutarate has not been demonstrated in experimental animals (17). It also appears that the synthesis of glutamine with ammonia utilizes as its source a small rapidly turning over pool of glutamate, rather than tricarboxylic acid cycle α -ketoglutarate (18).

Measurements of brain stores of high energy phosphate in acute ammonia intoxication in normal rats or in rats with portacaval anastomosis (PCA) have shown unaltered ATP contents in normal rats with hyperammonemia and a decline of ATP first after the onset of coma in rats with PCA. These findings indicate that cerebral dysfunction in ammonia intoxication is not due to primary energy failure (19, 20, 21, 22).

Looking further along the α -ketoglutarate-glutamate-glutamine pathway, depletion of glutamic acid has also been implicated in the pathogenesis of PSE. Glutamic acid is a putative excitatory neurotransmitter, although its importance in this role is not fully known (23). Reduced levels of glutamic acid have been observed in animals with experimentally induced hyperammonemia (19, 20, 24).

The detoxification of ammonia in brain yields glutamine. High levels of glutamine in cerebrospinal fluid (CSF) are found in patients with PSE (25), and in the brains of animals after hepatectomy (26), after portacaval anastomosis (27), and after ammonia intoxication (24). No parameter has correlated better with clinical severity of PSE than the spinal fluid glutamine (25). Any explanation of PSE must therefore take this fact into account. Glutamine applied iontophoretically is electrophysiologically inert in contrast to glutamic acid; however, it is taken up by nerve endings and may have some weak central depressant effect by itself, or may act by replacing other neurotransmitters (28).

α -ketoglutaramic acid, the product of transamination of glutamine, has also been implicated in the pathogenesis of PSE. α -ketoglutaramate is apparently a normal metabolite of glutamine and is found wherever glutamine is found (29). The causal relation of α -ketoglutaramate to PSE has not been established.

The importance of the α -ketoglutarate-glutamate-glutamine pathway in the reduction of elevated levels of ammonia in the brain has been demonstrated by two groups (30, 31). They used L-methionine-DL-sulphoximine, an irreversible inhibitor of glutamine synthetase, and demonstrated decreased ammonia toxicity, decreased synthesis of glutamine and increased ammonia concentrations after injection of

ammonium salts in rats. These studies strongly suggest that ammonia toxicity is a function of ammonia detoxification, not a consequence of ammonia concentrations per se.

Short-chain fatty acids

Short-chain fatty acids including butyrate, valerate, and octanoate are increased in the CSF and blood of patients with PSE (32, 33, 34). Fatty acid metabolism is disturbed in liver disease, and incomplete beta oxidation of long-chain fatty acids is probably the predominant source of the short-chain fatty acids (33). Except for the initial observations of Muto (34), attempts to show a clinical relation between high blood concentrations of fatty acids and PSE have not been successful (35). It is unlikely that short-chain fatty acids are solely responsible for PSE, but they have potential pathogenetic significance because of their relation to the possible toxicity of tryptophan and their augmentation of the toxic effects of ammonia and mercaptans (36).

Methionine and mercaptans

Methyl mercaptan (methanethiol) is elevated in blood in patients with liver failure and PSE (37). Methanethiol is formed in the gut from methionine. Normally, the mercaptans so formed are metabolized in the liver, but in patients with extensive portal-systemic shunting, these substances bypass the liver, accumulate in the blood, and are excreted in the breath and urine. Methanethiol inhibits brain microsomal ATPase, but the exact mechanism of the neurotoxic action has not been clarified (35).

Because of difficulties with the identification of the neurotoxic action of ammonia, short-chain fatty acids, and mercaptans, it has been suggested that a synergism between them is responsible for PSE (36). Indeed, reversible encephalopathy has been produced by the administration of ammonia and short-chain fatty acids and also mercaptans in rats. The ammonia and blood concentrations of these various metabolites, however, were far in excess of any such concentrations clinically seen in the blood of patients with PSE.

Amino acids

Disturbances of plasma amino acids in liver impairment have been described by several authors. A study in dogs have shown that in the total absence of the liver, all amino acids increase progressively in plasma except the branched-chain amino acids leucine, isoleucine, and valine, which decrease slightly (38). In acute liver failure in rats after two-stage hepatic devascularization, almost all plasma amino acids increase including the branched-chain amino acids (39). The liver failure after portacaval anastomosis in different experimental animals is accompanied by a characteristic distribution of plasma amino acids; the most consistent abnormality is an increase in the neutral amino acids phenylalanine, tyrosine,

tryptophan, methionine, and histidine, and a decrease in the branched-chain amino acids (40, 41, 42).

Investigations in humans with liver diseases and varying degree of PSE have demonstrated that plasma amino acid disturbances occur similar to those seen in experimental animals. In acute fulminant hepatic failure (viral hepatitis, toxic hepatic necrosis), the plasma concentrations of all amino acids except the branched-chain amino acids increase substantially (43, 44, 45). The branched-chain amino acids remain normal or decrease slightly.

Patients with PSE due to liver cirrhosis or after portal-systemic shunting of blood exhibit high plasma levels of methionine, phenylalanine, tyrosine, tryptophan, aspartate, proline, and cystine (the elevations are two-to-four fold), and low levels of the branched-chain amino acids (43, 46).

The mechanism underlying the alteration of plasma amino acids in liver impairment is not obvious. The origin of the raised plasma amino acids in fulminant hepatic failure is not certain. A part of the elevation may be attributable to the catabolic state, but on the basis of a close relation between plasma tyrosine and serum glutamic oxaloacetic transaminase levels, hepatic necrosis per se has been suspected to be responsible for the major part of the elevation (43).

The increase in aromatic and sulfur-containing amino acids in chronic hepatic insufficiency is most probably related to the catabolic state and the marked hyperglucagonemia which exists in the cirrhotic patient against a background of hepatic inability to metabolize increased gluconeogenic precursors (47, 48). The decreased concentration of the branched-chain amino acids is at least partially related to the hyperinsulinemia reported in this condition (49).

In an extensive study Fischer et al. (50) measured plasma amino acids in patients with PSE and suggested that the most significant changes were the elevation of the neutral aromatic and sulfur-containing amino acids and the depression of the branched-chain amino acids. They expressed this using a molar ratio:

Val + Leu + Ile

Phe + Tyr

the value of which is normally 3.0 to 3.5. In PSE it was found to be well below 1.0. They argued that this reduced ratio seemed critical in the induction of PSE and that its restoration to normal might be therapeutically beneficial.

Normally, there is competition between the aromatic amino acids - methionine, histidine, phenylalanine, tyrosine, and tryptophan - and the branched-chain amino acids - leucine, isoleucine, and valine - for passage through the blood-brain barrier. This has been

shown in experimental animals by measuring brain amino acids after diet-induced alteration of plasma amino acids (51) and by tracer studies of the blood-brain amino acid transport (52). It has also been demonstrated in humans by measuring brain extraction of amino acids on the basis of arterio-jugular venous concentration differences for amino acids (53).

Thus the alterations of plasma amino acid ratios with low levels of branched-chain amino acids in liver failure facilitates the entry of aromatic amino acids into the brain. Increases of methionine, phenylalanine, tyrosine, and tryptophan in brain and cerebrospinal fluid have been found in humans (45, 54) and laboratory animals with liver failure and PSE (40, 41, 55). Besides these changes the increase of glutamine predominates overwhelmingly as noted above.

In assessing the effects of portacaval anastomosis on the distribution of neutral amino acids between plasma and brain, it becomes apparent that the increase in brain concentrations of tyrosine, phenylalanine, and tryptophan cannot be explained simply on the basis of increases in plasma concentrations or altered competition in plasma. The magnitude of the concentrations of neutral amino acids seen in the brain are in excess of those one can calculate on the basis of plasma concentrations (55, 56). This suggests that there is an increased transport of neutral amino acids across the blood-brain barrier in liver failure. An increased brain uptake of radio-labeled neutral amino acids has been demonstrated in rats two and four weeks after portacaval anastomosis (40, 56). The presence of an increased transport activity is also supported by measurements of brain fractional uptake of neutral amino acids in humans with liver cirrhosis (53).

Tryptophan is unique among the neutral amino acids in that its transport into brain depends on both competition with other neutral amino acids and also on the concentration of tryptophan as the free amino acid, i.e. unbound to albumin. In a number of studies in animals with portacaval shunt, it appears that both free and total tryptophan play a role in determining the increase in not only the brain concentrations of the amino acid tryptophan, but also of its metabolites, serotonin and 5-hydroxyindoleacetic acid (57, 58). In chronic stable hepatic insufficiency in humans, one study showed that the increase in CSF tryptophan correlated both with increased plasma free tryptophan and decreased competition between tryptophan and the other neutral amino acids for blood-brain transport (54). In more severe models of liver failure in animals or in patients with acute hepatic failure, it appears that the decreased competition from other neutral amino acids is insufficient to explain the high brain level of tryptophan. Whereas the total tryptophan in the plasma may remain the same or decrease under these circumstances, there is a marked increase in plasma free tryptophan. This increase in free tryptophan appears to be sufficient to explain the increased penetration of tryptophan across the blood brain barrier. The increase of plasma free tryptophan depends on a decrease of serum albumin which is already decreased in chronic liver disease,

and on an increase of non-esterified fatty acids which displaces tryptophan from albumin (54).

The imbalance of plasma amino acids in hepatic failure and the increased transport of neutral amino acids into brain have been implicated in the etiology of PSE for several reasons:

1. The pattern of neutral amino acid changes in plasma, CSF and brain is a consistent finding in humans and experimental animals with liver failure and appears to correlate with encephalopathy (44, 45, 54).
2. Infusion of individual aromatic amino acids in the carotid artery in normal dogs has been reported to produce a reproducible coma (59).
3. A synthetic mixture of amino acids with low levels of methionine, phenylalanine, and tryptophan, and high levels of branched-chain amino acids has successfully reversed PSE produced in dogs by portacaval anastomosis or observed in cirrhotics requiring parenteral nutrition but intolerant to available standard amino acid solutions (41, 50).

Neurotransmitters

The subtle derangements seen in patients with chronic PSE suggest that altered neurotransmission may be taking place. Otherwise toxins would be insufficient to explain the subtle alterations in mood, drive, personality, sleep, and waking rhythm, which are recognized as so much a part of PSE. In 1971, Fischer and Baldessarini (60) proposed that under the circumstances of hepatic deterioration and shunting of blood around the liver, amines or their aromatic amino acid precursors bypassed hepatic inactivation, accumulated within the brain and led to replacement of putative adrenergic neurotransmitters with B-hydroxyphenylethylamines, and serotonin, which may replace the putative neurotransmitters norepinephrine and dopamine within the adrenergic systems. Since then, numerous laboratories have confirmed the relation between plasma octopamine and phenylethanolamine and the grade of encephalopathy (61,62). In another study of neurotransmitter contents in brains from patients dying with PSE, increased concentrations of tryptophan and serotonin and decreased levels of dopamine were found (63).

In rats with liver disease brain octopamine rose before plasma octopamine, suggesting that brain octopamine must be produced locally (64). Subsequent investigations have revealed that the origin of such B-hydroxyphenylethylamines and also serotonin can be traced back to their precursors phenylalanine, tyrosine, and tryptophan. Once within the brain, these aromatic amino acids result in derangements of the normal neurotransmitter profile including increased serotonin, decreased norepinephrine, and dopamine and increased B-hydroxyphenylethylamines (65). There are also increased

brain concentrations of taurine, an inhibitory putative neurotransmitter, and also decreased levels of glutamate and aspartate, which are excitatory putative neurotransmitters (24, 42). Several investigators have found a good correlation between neurotransmitter and amino acid changes in CSF and brain and PSE (66, 67).

This hypothesis has resulted in a series of therapeutic innovations which are based on correcting neurotransmission in PSE. L-dopa and bromocriptine appear to alter the neurotransmitter status within the central nervous system and have been used with success in some series (68, 69).

Another approach argues that if the plasma amino acid pattern is causally related to PSE, then normalizing the plasma amino acid pattern by infusion of special amino acid mixtures will result in awakening from PSE.

A series of centers have now demonstrated that, in chronic PSE with acute exacerbation, infusion of amino acid mixtures rich in branched-chain amino acids and low or lacking in aromatic amino acids results in awakening from PSE (50, 70, 71).

A subsequent study in dogs with portacaval anastomosis and PSE showed a decrease of CSF octopamine, phenylethanolamine, and 5-hydroxyindoleacetic acid after infusion of a branched-chain enriched amino acid solution concomitant with the improvement of PSE (41).

Encephalopathies in sepsis and uremia

The cerebral dysfunction occurring in patients with sepsis has not received much study.

In manifest sepsis with hypoperfusion, severe acidosis and hypoglycemia there may be several reasons for cerebral dysfunction. Early in the course of sepsis, however, many patients exhibit subtle derangement of cerebral function suggesting that altered neurotransmission may be taking place.

In a study of plasma amino acids in septic patients elevated levels of aromatic amino acids and normal or decreased levels of branched-chain amino acids were found (72). The derangement is similar to the one seen in patients with PSE. In another study, octopamine and phenylethanolamine increased in plasma in patients with septic encephalopathy (73). The frequent occurrence of metabolic encephalopathy in septic patients accompanied by changes in plasma levels of the aromatic amino acids and false neurotransmitters suggests that they may be causally related.

In five patients with overwhelming sepsis accompanied by a state of metabolic encephalopathy, an amino acid formulation enriched with branched-chain amino acids was administered (72). In these five patients, normalization of the plasma amino acid disturbance and reversal of encephalopathy was observed.

This observation would indicate that a mechanism similar to PSE may underlie the cerebral dysfunction in sepsis, but other factors may prevail.

The pathophysiology of uremic encephalopathy has not been clarified with certainty. As with PSE it seems to be a metabolic/neurophysiologic disorder at least initially without important structural lesions in the brain.

Several metabolic disturbances in uremia which may be correlated with cerebral dysfunction have been described. Among these are cerebral acidosis, decreased cerebral oxygen consumption, and depressed rates of cerebral glycolysis and of high-energy phosphate utilization (74). It is not clear, however, whether these findings bear upon the mechanisms of cerebral dysfunction in uremia.

Increased levels of octopamine in serum and urine have recently been demonstrated in patients with uremic encephalopathy (61,75). Recently also high CSF levels of tryptophan, 5-hydroxyindoleacetic acid and tyrosine have been demonstrated to occur in patients with chronic renal disease and encephalopathy (76). In a study of post-mortem human brains, from patients dying with uremic encephalopathy decreased concentrations of dopamine and increased levels of serotonin were found (63.) The pathogenetic significance of these neurotransmitter changes in uremia still remains uncertain, but they must be considered as potential mediators of the defective brain function in uremia.

AIM OF THE PRESENT STUDY

The most widely held concepts of PSE subscribe to the toxic mechanism of excess ammonia or to the altered amino acid balance and neurotransmitter changes. These two concepts have often been considered opposing mechanisms for the induction of PSE. As PSE clinically is accompanied by hyperammonemia, altered plasma amino acids, and elevated levels of false neurotransmitters, it is conceivable to believe that these changes are interrelated and causally linked to the pathophysiology of PSE.

The aim of the present study was to investigate whether there is any interrelation of hyperammonemia and plasma and brain amino acids.

Elevation of brain and CSF glutamine is a consistent finding clinically and experimentally in PSE, and is a reflection of hyperammonemia. Another purpose of the study was to evaluate the role of brain glutamine in PSE and its possible link to the increased transport of neutral amino acids across the blood-brain barrier.

It is an established clinical observation that substances involved in the production of PSE arise in the gut. Bacteria play a role in that altering gut bacteria flora is a cornerstone in therapy of PSE. It is not evident, however, whether gut bacteria play any role for the plasma and brain amino acid changes seen in PSE, and this was the purpose of the experiment in germ-free rats.

The encephalopathies of sepsis and uremia show clinically several similarities with PSE. Therefore plasma and brain amino acids and blood-brain amino acid transport were studied in experimental models of sepsis and uremia to investigate whether a common pathophysiological pathway of these conditions is present.

METHODOLOGICAL CONSIDERATIONS

Animals

Conventional rats

Male Sprague-Dawley rats weighing 200-300 g were used (Charles River Laboratories, Wilmington, MA). The rats were fed with Purina rat chow ad libitum (Ralston Purina Co., St. Louis, Mo.) and tap water was available at all times. The animals were housed 4 per cage. Lights were on between 7 a.m. and 7 p.m. daily.

Germ-free rats

Germ-free male Sprague-Dawley rats weighing 200-225 g were used in one experiment (IV) (Charles River Laboratories, Wilmington, MA). The animals were housed in a germ-free isolator (G.F. Supply Division, Standard Safety Equipment Co., Palatine, Ill.,) They were fed ordinary rat chow, (5010-C Autoclavable, Tasty Foods, Cincinnati, OH.,) and water which had been steamsterilized. From arrival to sacrifice, the germ-free rats were carefully monitored bacteriologically. Samples from the isolator and from detritus or stool of the animals were taken for culture twice weekly or each time an item was passed in or out of the isolator. The samples were placed in freshly boiled and cooled thioglycolate and incubated at 37°C and at room temperature for 24 and 48 hours. Periodic cultures of the gloves, cage contents and water supply were also performed. There was no detection of aerobic or anaerobic bacteria in any sample throughout this experiment.

Experimental models

Portacaval anastomosis

The rats were anesthetized with ether. A portacaval anastomosis was created by the recently described method (77). Using clean, but not sterile technique, the abdominal wall was opened on the midline; the portal vein was exposed, dissected, and ligated. The clamped portal vein was then cut free and fed through a small piece of Teflon tubing previously sculptured to form a "button" of the type used for vascular anastomoses. The vein was everted over the button and secured with a silk ligature; the vein-button assembly was then anastomosed to the partially clamped vena cava with a purse string suture. After the removal of the clamps, the patency of the shunt was checked by the rapid return of normal color to the bowel. At the end of the operation, 10 - 15 ml saline was instilled in the peritoneal cavity. When using this technique the perioperative mortality was less than 5 percent.

Animals undergoing this operation show a depression of food intake postoperatively for 5 to 7 days. By the tenth day, food intake

usually achieves or surpasses preoperative levels and the animals regain their preoperative weight. This pattern has been observed in this laboratory and in others for a long time.

In experiments of this study, daily food intake and body weights were not measured. Animals with a body weight equal to or lower than the preoperative weight were excluded.

The germ-free rats were operated with the same technique inside the germ-free isolator. These animals were anesthetized with ketamine hydrochloride (Ketaset^R) 35^R mg/kg B.W. i.m. with the addition of local anesthetics (Xylocain 1 %) 1 - 2 ml infiltrated in the abdominal wall.

A sham-operation consisted of laparotomy with dissection of portal vein and inferior vena cava and clamping of portal vein for five minutes.

All animals were operated and sacrificed at the same time: 10 a.m. - 2 p.m.

Administration of ammonium salts

Normal unoperated rats were injected intraperitoneally with a 1M solution of ammonium acetate or sodium acetate in a dose of 5 mMol/kg body weight. Three doses were given at 30-minute intervals. The injections made most of the animals drowsy, but no convulsions were seen. Thirty minutes after the last injection, the animals were sacrificed by decapitation and blood was collected from the cervical wound into heparinized chilled beakers. Brains were blotted to remove adhering blood, hemisected sagittally and frozen on dry ice.

Abdominal sepsis

Nonstarved rats were used. Abdominal sepsis was produced according to the technique described by Wichterman et al.(78). Under light ether anesthesia, the cecum was ligated just below the ileocecal valve, and the ligated cecum was punctured twice with an 18 gauge needle. Sham operation consisted of laparotomy with handling of cecum but without ligation or puncture. Postoperatively, all animals were injected subcutaneously with saline (10 ml/100 g body weight) every 6 hours in order to maintain normal circulation.

The animals were sacrificed as above; one group 10 to 12 hours after operation ("early sepsis"); another group 22 to 24 hours ("late sepsis"). Blood cultures and abdominal cultures taken at death from septic animals all showed growth of a mixed fecal flora.

Uremia

Non-fasted male rats were used in the experiments. Chronic uremia was produced by unilateral nephrectomy and contralateral segmental renal infarction under light ether anesthesia (79). The surgery resulted in a 75 - 80 percent ischemic infarction.

Acute uremia was produced according to the model described by Iaina et al. (80). Under light ether anesthesia, a unilateral nephrectomy was performed and the other kidney was stripped of all the surrounding connective tissue. The renal artery was then isolated and clamped for 70 minutes.

Animals with chronic uremia were sacrificed 14 days after operation, as described above, and rats with acute uremia after 24 hours.

All operations and sacrifices were performed between 9 and 12 a.m.

Biochemical assays

Amino Acid Analysis

Blood was collected from the cervical wound into chilled heparinized beakers and immediately transferred to glass tubes, which were stoppered and placed in an ice bath. The blood was then centrifuged at 4°C to separate plasma, which was either analyzed immediately or frozen at -70°C . No plasma was frozen longer than 2 weeks before analysis. Plasma was deproteinized by mixing with an equal volume of 7.5 percent sulfosalicylic acid (SSA) pH 1.7, containing an internal standard (norleucine or thienylphenylalanine). After vortex mixture, the samples were centrifuged at $30,000 \times g$, 4°C , for 20 minutes. The supernatant was passed through a 0.45 micron filter (Millipore) and analyzed directly.

Brains were blotted to remove adhering blood, hemisected sagittally and frozen on dry-ice. One half of each brain was homogenized in three volumes (3 x weight) of 5 percent SSA, pH 1.4 containing an internal standard and treated further as above. Each brain sample was analyzed twice, once as the original homogenate and again after 1:25 dilution in lithium citrate buffer, pH 2.2. The latter dilution was necessary for accurate quantitation of glutamine in an abbreviated analysis program.

Free amino acids of plasma and brain of each animal were then analyzed using a Beckman 121-MB automatic amino acid analyzer and lithium citrate buffers. (Beckman Instruments, Palo Alto, CA).

Other plasma assays

All assays were performed in the blood collected from the cervical wound.

Plasma ammonia was determined immediately after sacrifice by the glutamate dehydrogenase reaction with a centrifugal analyzer (CentriChem, Union Carbide, Inc.) (81). Albumin was determined by a dye-binding method using bromocresol green. Glucose was assayed

using a kinetic glucose-oxidase method of JL 919 at 37° C. (Instrumentation Labs, Inc.). BUN was determined with a coupled urease - glutamate dehydrogenase kinetic assay at 37° C on the JL 919.

Blood-brain transport of amino acids

Studies of the blood brain transport of different amino acids were performed according to the technique of Oldendorf (82). This study was performed in all experiments except in the experiment with ammonia-administration (III).

The rats were anesthetized with pentobarbital (Sodium Pentobarbital R) 35 mg / kg B.W. intraperitoneally. A midline incision in the neck was immediately made, the left common carotid artery was exposed and cannulated with a 27 gauge needle. A rapid injection of 0.2 ml of a mixture containing 1.0 μ Ci of $^3\text{H}_2\text{O}$ and 0.2 μ Ci of ^{14}C -labeled amino acid in Krebs-Ringer buffer adjusted to pH 7.4 with 10 mM Hepes was made without occluding blood flow. Exactly 15 seconds after the injection, the rat was decapitated and the brains were immediately removed. Blood from the cervical wound was collected for biochemical assays.

A sample of brain tissue ipsilateral to the injection and rostral to the midbrain was extruded through a 20 gauge syringe needle into 1.0 ml NCS tissue solubilizer. (Amersham/Searle, Arlington Heights, Ill.)

The brain tissue samples and the injection solutions were analyzed by double-isotope liquid scintillation counting (Packard Tri-Carb). The brain content of the rapidly transported amino acid was compared to the uptake of the freely-diffusable reference $^3\text{H}_2\text{O}$.

Brain uptake index (BUI) was calculated as:

$$\frac{(\text{dpm } ^{14}\text{C}/\text{dpm } ^3\text{H}) \text{ brain}}{(\text{dpm } ^{14}\text{C}/\text{dpm } ^3\text{H}) \text{ injectate}} \times 100$$

All radioactive substances were obtained from New England Nuclear Corporation (Boston, MA.). All other chemicals were from Sigma Chemical Co. (St. Louis, Mo).

Inhibition of blood-brain phenylalanine uptake by glutamine

In order to obtain an estimate of the Michaelis-Menten constant, K_m , of glutamine for the neutral amino acid transport of the blood-brain barrier, the effect of various concentrations of glutamine on the in-vivo brain uptake of radiolabeled L-phenylalanine was studied. Pardridge (52) has shown that the K_i of transport of one neutral amino acid for another, i.e., the concentration at which 50 percent inhibition of the saturable component of uptake occurs, is close to the K_m of transport determined by self-competition for uptake.

Normal unoperated rats were used and the experiment was performed as above.

Carotid injection solutions contained in 0.2 ml, 0.2 μCi L-(^{14}C) phenylalanine ($2 \times 10^6 \text{ M}$), and 1.0 μCi (^3H) water, as freely diffusible reference in Ringer's solution buffered to pH 7.4 with 10 mM Hepes were used. Solutions containing unlabeled glutamine were prepared as above but with 2 mM, 8 mM, 10 mM, 20 mM and 40 mM L-glutamine (Eastman Kodak Co., Rochester, N.Y.).

To maintain constant osmolarity of the solutions, NaCl was reduced in proportion to the addition of glutamine.

The calculation of K_i of glutamine from a double reciprocal transformation of the data was performed as described by Pardridge (52).

Plasma competitor functions

In a study of diet-induced changes of plasma and brain neutral amino acids, Fernstrom and Faller demonstrated in normal rats that the brain level of any one of these amino acids could not reliably be predicted by an alteration in its serum concentration (51). To assume that the level of an amino acid in brain should reflect its concentration in blood is to infer that amino acid uptake into brain proceeds largely by mass action or diffusion. It has repeatedly been shown, however, that amino acids are transported into brain by carrier mechanisms that are charge and size specific (83). In this study, it was shown that the brain concentration of a particular neutral amino acid correlates well with the molar ratio in plasma of that neutral amino acid to the sum of the concentrations of the other plasma neutral amino acids competing for transport. The study also showed that a somewhat better correlation was obtained if the Michaelis-Menten constant, K_m , of transport of each neutral amino acid was taken into account. We chose to express plasma neutral amino acid competitor ratios taking into account the transport K_m 's of the neutral amino acids as determined in vivo for blood-brain transport in normal rats (84) and we presumed that these constants were not changed in the disease-states studied. The neutral amino acids considered in competitor calculations were threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, and histidine. Tryptophan, which also competes for blood-brain neutral amino acid transport, was not considered because the fraction of plasma total tryptophan which participates in competition for transport, cannot be reliably determined, and because rats in all experiments except uremia showed only slight differences in plasma total tryptophan. Therefore, disregarding tryptophan in competition calculations should induce only a slight error.

Plasma competitor functions were calculated as the brain influx rate used by Fernstrom and Faller (51), with the modification that the V_{max} of transport, determined by Pardridge and Oldendorf (84) for normal rats, was not included explicitly in the calculation. That is, plasma competitor functions (PCF) were calculated as follows:

$$PCF_x = \frac{(AA_x)}{K_{m_x} \left[1 + \sum \frac{(AA)}{K_m} \right] + (AA_x)} \times V_{max}$$

Where:

(AA_x) = plasma concentration of a neutral amino acid

K_{m_x} = transport K_m of a neutral amino acid

(AA) = plasma concentrations of competing neutral amino acids

K_m = transport K_m of competing neutral amino acids

V_{max} = transport V_{max} -unspecified

The K_m 's of transport for each neutral amino acid, from Pardridge and Oldendorf (84), were as follows: threonine - 730 nmol/ml; valine - 630 nmol/ml; methionine - 190 nmol/ml; isoleucine 330 nmol/ml; leucine - 150 nmol/ml; tyrosine - 160 nmol/ml; phenylalanine - 120 nmol/ml; histidine - 280 nmol/ml.

Plasma competitor functions of all neutral amino acids were calculated for every animal in the experiments.

Isolated brain capillaries

In the study of abdominal sepsis, the transport of different amino acids in vitro in isolated brain capillaries was studied. The capillaries were isolated from brain essentially as described by Hjelle et al.(85). The isolated vessels were mostly branching capillary segments with some small arterioles and venules. The isolated capillaries were then incubated with 20 μ Ci of 14 C-labeled amino acid. The uptake of 14 C-labeled amino acid was followed at 37°C; a 600 μ liter aliquot was withdrawn at different times up to 300 minutes. Each aliquot was immediately pipetted on a 44 μ m pore size nylon sieve on a vacuum manifold and washed three times with 5 ml of cold buffer. The sieve with the retained microvessels was placed in a tube containing 1.8 ml of 1.0 N sodium hydroxide and remained overnight at room temperature. Each tube was then sonicated for 1 minute. Portions were taken either for protein determination by the method of Lowry et al. (86), using bovine albumin as standard or for determination of radioactivity in a liquid scintillation spectrometer (Packard Tri-Carb).

The data were expressed as the distribution ratio (inside/outside).

Statistics

The data obtained were subjected to statistical analysis using the student's t-test for unpaired observations. All values are given as the mean $\pm$ S.E.M.

In evaluation of the role of brain glutamine in blood-brain neutral amino acid transport after PCA (II), analysis of Pearson product-moment correlation was performed to determine the relation (a) between plasma competitor functions and brain neutral amino acid concentrations for each of the eight neutral amino acids in control rats and in all rats with PCA; (b) between brain glutamine and brain neutral amino acid concentrations for the same neutral amino acids in control rats and rats with PCA; and (c) between plasma competitor functions and brain glutamine for the same neutral amino acids, considering rats with PCA alone and rats with PCA and controls taken together.

Partial correlations were performed in order to determine the relation between any two variables alone with the relating effects of the third variable taken out of both variables (87). Multiple correlations were calculated to determine the relationship between brain neutral amino acid concentrations and the combined effects of plasma competitor functions and brain glutamine, taken together.

RESULTS AND DISCUSSION

Brain neutral amino acids, plasma neutral amino acid competitor functions and brain glutamine after PCA (I, II)

After an initial rise in most plasma neutral amino acids at seven days after PCA, plasma phenylalanine, tyrosine and histidine remained elevated for periods up to 70 days. Plasma leucine, isoleucine, and valine were lower than normal after PCA. In brain, phenylalanine, tyrosine, histidine, and methionine were markedly elevated at all times after PCA, and their concentrations in brain far exceeded the concentrations in plasma. In contrast, brain levels of threonine, leucine, isoleucine, and valine remained near normal after PCA.

The alteration of plasma neutral amino acids competing for blood-brain transport after PCA favours the entry of phenylalanine, tyrosine, histidine, and methionine into brain, but their brain levels are higher after PCA than can be accounted for by altered competition at the blood-brain barrier. Indeed, increased blood-brain transport after PCA has been shown for neutral amino acids (40, 56) that could explain the discrepancies, but the mechanisms underlying this increase of transport activity are not known. After PCA, the concentration of glutamine in brain is markedly increased, and the rate of efflux of glutamine from brain is also increased (20, 24). Glutamine is thought to be a weakly competing substrate for the blood-brain neutral amino acid transport system (83), and it is reasonable to assume that the efflux of glutamine from brain is mediated, at least in part, by the neutral amino acid transport system of the luminal capillary surface. The properties of this transport system are those of the L-system for neutral amino acid transport which is Na^+ -independent and demonstrates exchange (88).

Therefore, high brain glutamine concentrations after PCA could raise the influx of neutral amino acids from blood by accelerating exchange transport via the neutral amino acid transport system.

In order to examine the relation of brain glutamine concentration to plasma and brain neutral amino acid concentrations, analysis of Pearson product - moment correlation was performed to determine the relation between plasma competitor functions, brain neutral amino acid concentrations and brain glutamine.

The correlation between plasma competitor functions and brain neutral amino acid concentrations in normal rats yielded statistically significant values of the correlation coefficient (r) for threonine, isoleucine, leucine, tyrosine, and phenylalanine. Values of r were generally lower in rats with PCA, reflecting a weaker correlation between plasma competitor function and brain neutral amino acid concentration. For tyrosine and phenylalanine, the data from control rats and rats with PCA yielded excellent, significant correlations (Figure 1).

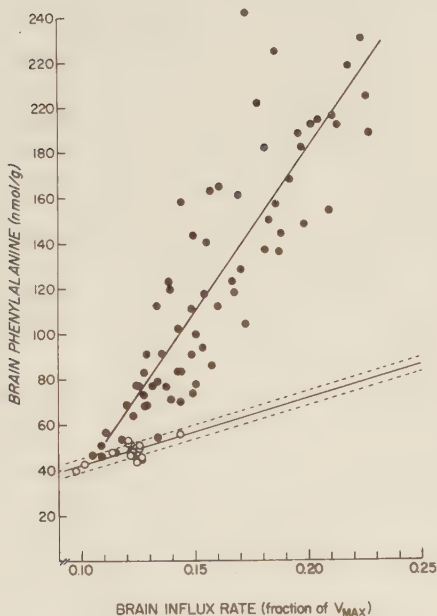
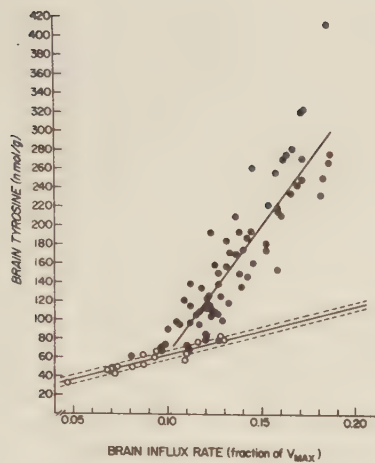


Figure 1. The relation between the calculated brain tyrosine/phenylalanine influx rates, based on plasma competitor functions, and brain tyrosine/phenylalanine levels in control (o) and PCA (●) rats. The dashed lines are placed 2 S.D. above and below the control regression line.

The slopes of the regression lines for rats with PCA, however, were markedly steeper than those for controls. This difference in slopes implies a difference in the kinetic constants, K_m or V_{max} , describing transport phenomena in the two groups. It is reasonable to suppose that transport K_m 's are unchanged after PCA as this parameter presumably reflects physical properties of the transport system and of the neutral amino acids themselves and as studies in vitro of brain microvessels isolated from rats with PCA and controls show no differences in the K_m 's of uptake of neutral amino acids (89). Therefore, it may be concluded that PCA affects the V_{max} of uptake.

The correlations between brain glutamine concentration and brain neutral amino acid concentration in control rats were all weak. In rats with PCA, the brain concentration of tyrosine, phenylalanine, histidine, threonine and methionine correlated significantly with the brain glutamine concentration, and the correlation was better (higher r) than with the appropriate plasma competitor function. The correlations between plasma competitor functions and brain glutamine were all non-significant in control rats. When rats with PCA were considered alone or together with the controls, significant correlations, both positive and negative, were found. It is highly unlikely that either of these two variables can have a direct effect on the other. Because no likely causal link exists between these two variables, it is probable that another, unmeasured factor affects both plasma competitor functions and brain glutamine independently. Brain glutamine concentration after PCA is probably strongly influenced by the degree of portal-systemic shunting and thus the blood ammonia concentration (24, 27). Similarly, the specific increases and decreases of various neutral amino acids in plasma after PCA are probably proportional to the degree of portal-systemic shunting. Consequently, plasma competitor functions and brain glutamine will be strongly correlated by their common dependence on hepatic function.

It was shown that brain glutamine and plasma competitor functions were not independent variables, therefore partial correlations were carried out to determine for each neutral amino acid the separate effects of the plasma competitor function or of the brain glutamine on the brain neutral amino acid concentrations. In this analysis the effects of the correlations between plasma competitor functions and brain glutamine could be taken out. It showed that brain glutamine concentration was the better predictor of brain neutral amino acid concentrations.

To determine whether the effects of plasma competitor functions and brain glutamine concentration acting together gave a stronger correlation with brain neutral amino acid concentration than did either factor separately, multiple correlations were calculated. In all cases, the correlation coefficients obtained from the multiple correlations equalled or exceeded those obtained in the partial

correlations or those for rats with PCA in the two single factor correlations in which brain neutral amino acid concentration was one of the variables. For tyrosine, phenylalanine, and histidine, highly significant correlations were obtained. The results of this analysis show that the combined effects of plasma competitor functions and brain glutamine concentration predicted brain neutral amino acid concentrations better than did either factor separately. This suggests that the effects of plasma competitor functions and brain glutamine concentration on brain neutral amino acid levels are separate and additive.

This conclusion is further supported by the excellent correlation between brain glutamine concentration and the differences between the observed and predicted brain neutral amino acid concentrations (Figure 2). The linear regression equations describing the relation between brain neutral amino acids and plasma competitor functions in normal rats (Figure 1) were used to generate the "expected" brain neutral amino acid concentrations by substitution of the plasma competitor functions of rats with PCA. For each rat, the difference between the observed brain concentration of each neutral amino acid and the predicted concentration was calculated. The relation between this difference and the brain glutamine concentration was determined. With the exception of valine, positive, significant correlations were obtained for all neutral amino acids. In these correlations the statistical relation between brain glutamine and brain neutral amino acids improved when the variability in the latter due to plasma competitor functions had been explicitly removed.

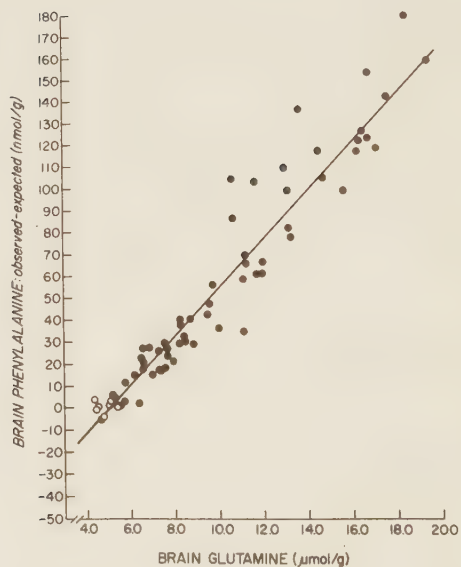
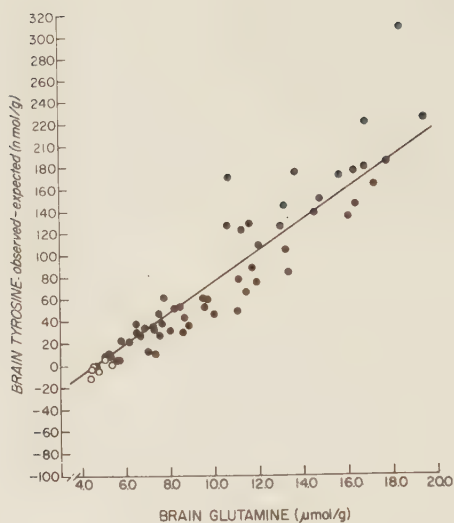


Figure 2. Relation between brain glutamine concentration and the differences between the observed and predicted brain tyrosine/phenylalanine concentrations.

The present study demonstrates a close relation among the brain concentrations of many neutral amino acids, plasma neutral amino acid competitor functions, and the brain glutamine concentration. The results support the conclusion that after PCA the brain concentrations of many neutral amino acids are strongly influenced by the combined effects of plasma competitor functions and brain glutamine concentration. The strong correlation between brain glutamine and brain neutral amino acids suggests that glutamine exerts some effect on blood-brain neutral amino acid transport. If glutamine is to influence the blood-brain transport of neutral amino acids, it must be shown that glutamine utilizes the same transport system as the neutral amino acids and competes with them for transport.

Inhibition of brain uptake of phenylalanine by glutamine (II)

Although glutamine is a neutral amino acid, it has never been shown to interact with the same carrier as the one used by the other neutral amino acids for blood-brain transport. Addition of glutamine to the carotid injection solution reduced the brain uptake of ^{14}C -phenylalanine in a concentration dependent manner. If it is assumed that this represents competitive inhibition for blood-brain transport, the K_i (8.48 mM) obtained by double-reciprocal transformation of the data (52) may be taken as a reasonable approximation of the K_m of transport of glutamine.

It thus appears that glutamine competes with other neutral amino acids for the same carrier-system at the blood-brain barrier. The apparently high K_m of glutamine indicates that it has a relatively low affinity for the neutral amino acid transport system of the blood-brain barrier. This low affinity for transport combined with low plasma concentrations of glutamine in liver failure imply a minor transport of glutamine from blood to brain. Glutamine is manufactured locally in the brain from glutamic acid and ammonia. In disease-states with hyperammonemia, brain glutamine rises markedly, far exceeding other neutral amino acids, and this may be important in regulating transport of glutamine and the other neutral amino acids from brain to blood.

Increased synthesis of glutamine in brain is one effect of hyperammonemia, which seems to be of importance in affecting brain neutral amino acids and in the etiology of PSE. It is not known, however, if hyperammonemia also has any effects on plasma amino acids.

Effects of ammonia-administration on plasma and brain amino acids (III)

The administration of ammonium-acetate in a dose of 5 mM/kg B.W. to normal rats resulted in a moderate rise in plasma ammonia at the time of sacrifice to about the same level as seen after PCA.

Almost all plasma amino acid concentrations were decreased. This was most pronounced for alanine, glycine, serine, threonine and the branched-chain amino acids valine, isoleucine and leucine. There were modest increases of plasma citrulline, histidine, methionine, phenylalanine, taurine, and tryptophan.

The etiology of the amino acid changes in plasma induced by ammonia is not obvious. Other studies have indicated that the changes may be related to disturbances of glucose-metabolism, often seen in patients and animals with portal-systemic shunting and hyperammonemia. Patients with portal-systemic shunting have high glucagon levels and the degree of hyperglucagonemia correlates with the degree of ammonia intolerance (48, 90).

Strombeck et al.(91) demonstrated in intact dogs infused with ammonium-salts after 2 hrs an increase of plasma glucagon levels to 20 times resting levels, although in this study plasma ammonia was raised five times compared with the present study.

It thus seems as if high blood ammonia, either endogenously or exogenously derived, stimulates glucagon secretion.

The promotion of gluconeogenesis increases glucose synthesis from amino acids and, as a consequence, increases the production of ammonia. This may be one reason behind the hyperinsulinemia frequently observed in cirrhotic patients during portal-systemic encephalopathy with increased blood ammonia levels (90). Another cause for the hyperinsulinemia in cirrhotic patients may be the peripheral insulin resistance seen in these patients, which is probably aggravated by hyperammonemia (92, 93). In the study by Strombeck et al.(91), plasma insulin increased to 10 times resting levels in 4 hrs after ammonia infusion began.

The increased insulin levels in these instances maintain blood glucose within normal limits, but stimulate the uptake and therefore the catabolism of branched-chain amino acids by muscle (49).

When liver function is compromised, phenylalanine, tyrosine, and methionine rise in plasma because protein breakdown is favoured by a low blood insulin/glucagon ratio and because the liver is the main site of catabolism of these amino acids (47). In this experiment in animals with normal liver function, phenylalanine, tyrosine and methionine remain within normal limits in plasma, but the branched-chain amino acids decrease. The reasons for the decrease in plasma levels of alanine, glutamate, glycine, lysine, ornithine, proline, and serine in the animals after ammonia-administration are not obvious. The decrease of alanine, glutamate, the branched-chain amino acids, may reflect ammonia-detoxification by the muscle; the

decrease of the other amino acids may be an effect of glucagon, which has been shown to have a hypoaminoacidemic effect involving virtually all amino acids (94), although glucagon was not measured in this experiment.

All neutral amino acids except threonine increased in brain after ammonia load. There were also moderate increases of brain alanine and citrulline and a three-fold increase of glutamine.

The establishment of the plasma amino acid pattern with low levels of branched-chain amino acids and high or normal levels of aromatic amino acids after ammonia administration will facilitate the transport into brain of the aromatic amino acids. The altered competition among plasma neutral amino acids is reflected in altered plasma competitor functions in rats after ammonia administration (Table I).

Amino Acid	Plasma Competitor Function			Brain Amino Acid		
	NH ₄ (11)	Na (9)	% ^x)	NH ₄	Na	% ^x)
Valine	.045 ± .003	.060 ± .002 ⁺	75	135 ± 16	87 ± 7 [*]	155
Isoleucine	.049 ± .001	.062 ± .001 ⁺⁺	79	45 ± 3	34 ± 2 [*]	132
Leucine	.184 ± .006	.219 ± .003 ⁺⁺	84	96 ± 8	72 ± 4 [*]	133
Tyrosine	.105 ± .003	.099 ± .004	106	112 ± 4	48 ± 4 ⁺⁺	233
Phenylalanine	.146 ± .004	.130 ± .004 [*]	112	95 ± 2	46 ± 1 ⁺⁺	206
Histidine	.059 ± .001	.038 ± .001 ⁺⁺	155	113 ± 5	53 ± 2 ⁺⁺	213

Table I. Plasma competitor functions and brain neutral amino acids in rats injected with ammonium-acetate or sodium-acetate. Results are given as mean ± S.E.M. for numbers of rats in parentheses. * p < 0.05, p < 0.01, and p < 0.001 compared with control values. ^x)%, value for NH₄ expressed as percentage of control.

The increases in brain neutral amino acids are larger, however, than can be predicted from altered plasma amino acid competition. Thus, the rise in brain concentration of neutral amino acids is due not only to decreased competition for entry into the brain, but also to other factors which presumably increase the transport activity or impede the transport out of the brain.

After ammonia load, brain glutamine and alanine rose significantly. The rise in brain glutamine reflects ammonia-detoxification by the brain, and an increase in brain and CSF glutamine is a consistent finding in patients and animals with PSE and in animals after ammonia load (24, 25). The rise in brain alanine probably also illustrates ammonia-detoxification by the brain (22).

CSF-concentrations of glutamine have shown a good correlation with the grade of encephalopathy both in dogs (41) and humans (25). Glutamine is a neutral amino acid, and the high brain glutamine repeatedly occurring with hyperammonemia due to hepatic failure or portal-systemic shunting may be coupled to the increased blood-brain transport of other neutral amino acids in these conditions by an exchange transport, as discussed above.

The results of this experiment suggest that hyperammonemia may contribute to the plasma and brain neutral amino acid changes in liver disease.

Plasma and brain amino acids after portacaval anastomosis in germ-free rats (IV)

In patients and animals with liver failure, PSE is always accompanied with a disturbed nitrogen metabolism. Whether ammonia, amine or amino acid metabolism is causally related to etiology, gut bacteria have been repeatedly implicated both in etiology and by the manipulation of gut flora (neomycin) in therapy of PSE (6, 7, 15, 35). Although previous studies have demonstrated that the metabolism of ammonia is not grossly different in germ-free animals (10), no study has been directed to the metabolic changes in neutral amino acid metabolism and blood-brain barrier activity changes seen after portacaval shunt in germ-free animals.

The elevation of plasma ammonia after PCA seems unaffected by the presence of gut bacteria. The elevation of plasma ammonia after PCA was slightly smaller in germ-free rats, but the germ-free control rats had higher ammonia levels than conventional controls. The results from this and other studies indicate that the hyperammonemia seen after PCA does not reflect ammonia synthesis by gut bacteria, but rather endogenous metabolic processes, e.g., in small intestine, kidneys, and muscle (11, 12, 13, 14).

The role of gut bacteria in promoting protein breakdown, enhancing amino acid absorption and thus contributing to the disturbance of plasma amino acid pattern seen in liver failure if any, is unknown.

The plasma and brain amino acids in germ-free control rats did not differ from conventional rats. The conventional rats had slightly higher plasma levels of hydroxy-proline, ornithine, proline, and threonine, and this may be a reflection of bacterial production of these amino acids in the gut (95).

After PCA, both groups of rats demonstrated similar changes of plasma amino acids. Specifically, the changes of neutral amino acids with low levels of branched-chain amino acids and high levels of aromatic amino acids were not a reflection of bacterial protein or amino acid metabolism. Both groups of shunted rats also exhibited similar elevations of aromatic amino acids in brain.

The increased transport of neutral amino acids into brain after PCA also seemed to be unaffected by gut bacteria. The brain uptake index for tryptophan was increased after PCA to the same degree in germ-free and conventional rats.

The plasma competitor functions of tyrosine, phenylalanine and histidine were all increased in shunted rats. These changes reached the same levels in germ-free and conventional rats. The relation between plasma competitor function and brain neutral amino acid concentration, as shown in the figure for tyrosine, demonstrates a higher brain level in shunted rats than can be predicted from

plasma competition in control rats (Figure 3). This is also an indication of the increased transport of neutral amino acids into brain after PCA.

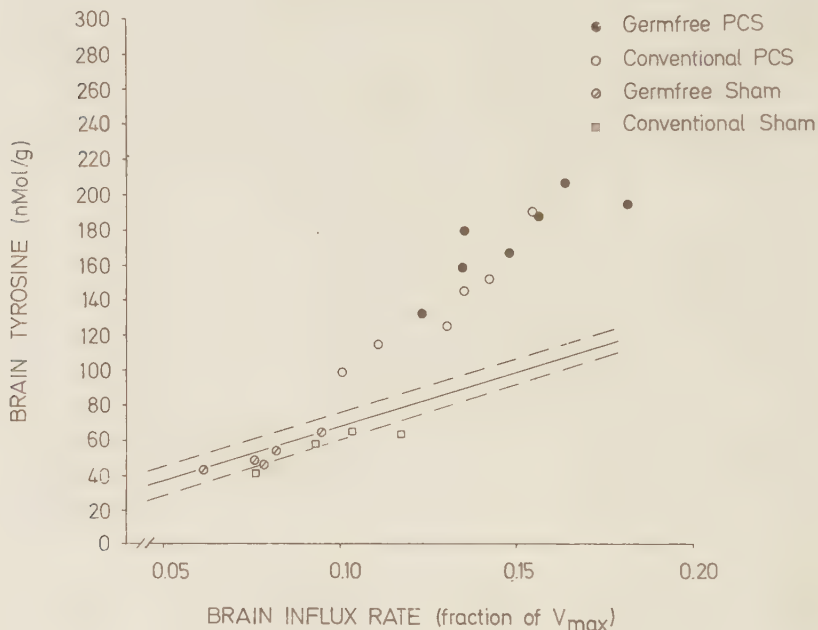


Figure 3. The relation between brain tyrosine and the brain influx rate (fraction of V_{max}). The dashed lines are placed 2 S.D. above and below the control regression line.

The proposed mechanism for the increased transport activity after PCA by an exchange with brain glutamine may occur independent of bacterial presence in the gut. All factors that are believed to be involved in raising brain neutral amino acids and play a role in the pathophysiology of PSE in rats--that is hyperammonemia, altered plasma amino acid ratios and high brain glutamine--are present to the same extent in germ-free and conventional rats after PCA.

Plasma and brain amino acids in abdominal sepsis (V)

We have performed this model of abdominal sepsis as described by Wichterman et al. (78). Ten to twelve hours after cecal ligation and puncture, most rats appeared only mildly ill. However, blood cultures obtained from such rats all showed growth of a mixed fecal

flora. These rats are referred to as "early septic". Recent studies from the laboratory of Wichterman, Baue and Chaudry (96) have shown that rats in early sepsis show features associated with a hyperdynamic circulation and hyperglycemia and could resemble what has been described as "high output sepsis" in patients (97).

Twenty to twenty-four hours after operation the condition of the rats had deteriorated; the animals moved about less, they became unpreened, progressively lethargic and allowed themselves to be laid on their backs, on the table, without objection. Rats at this stage are referred to as "late sepsis". In the studies by Wichterman et al.(96), these animals were shown to be hypodynamic and hypoglycemic.

In our experiment, blood-pressure was not monitored. Rats in early sepsis were normoglycemic, whereas the late septic rats had hypoglycemia. The difference in the early septic group from the original report can be due to more vigorous hydration in our experiment.

In both septic groups, there were changes in plasma amino acids with a decrease of the urea precursors arginine and proline and also the gluconeogenic precursor glycine, whereas alanine remained normal. These results differ somewhat from results in humans, where all of these amino acids except arginine are usually elevated (72). In other animal models of sepsis, a reduction in total plasma free amino acids has been shown however (98). The late septic group had a reduction of all the branched-chain amino acids. This is described by others, both in experimental animals and in patients, and probably represents a peripheral energy fuel deficit and increased oxidation by skeletal muscle (72, 98, 99). The increased muscle proteolysis and decreased hepatic amino acid uptake produce an increase in phenylalanine and tyrosine (72). The plasma amino acid pattern, especially in the rats with late sepsis, is similar to that seen in rats or humans with PSE.

In brain, most of the neutral amino acids were increased. Phenylalanine, histidine, and tyrosine, and also in the late septic group, some of the branched-chain amino acids showed the largest increases. Most of the other amino acids were unchanged in brain. The sharp increases of phenylalanine, histidine, and tyrosine corresponded well with the findings after PCA. The elevated levels of the aromatic amino acids in brain could partly be explained by a decreased competition among neutral amino acids by the decrease of branched-chain amino acids, at least in the late septic group. However, there seemed also to be an increased blood-brain transport of neutral amino acids in sepsis. Brain levels of phenylalanine, histidine, and tyrosine were higher in most cases than could be predicted from altered plasma competition among neutral amino acids. This is shown for phenylalanine in Figure 4, and is in analogy with findings after PCA, although to a lesser degree.

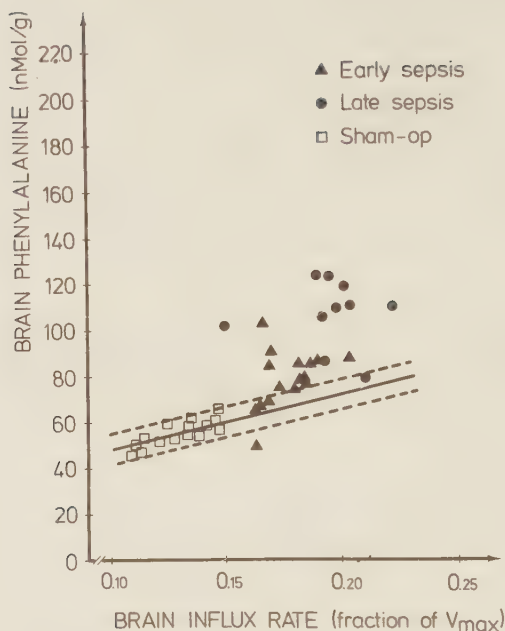


Figure 4. Relation between brain phenylalanine and brain influx rate (fraction of V_{max}) in septic and sham-operated rats. The dashed lines are placed 2 S.D. above and below the control regression line.

The presence of an increased blood-brain transport of neutral amino acids is also supported by the findings of increased brain uptake indices for phenylalanine in early sepsis and for tryptophan and tyrosine in late sepsis. The increase seemed to be specific for the neutral amino acid carrier system, as the brain uptake index for lysine, a basic amino acid, was normal.

The study of the transport in vitro in isolated brain capillaries from septic rats also lends support to the presence of a specific derangement of the neutral amino acid carrier system. This preparation had an increased uptake by the capillaries from septic rats of leucine and tyrosine, but a normal uptake of lysine. These results resemble findings in rats with PCA (89).

The results of this study indicate that septic encephalopathy may involve a basic underlying disturbance of plasma and brain neutral amino acids and of the blood-brain transport of neutral amino acids, similar to PSE. A resultant disturbance of neurotransmitter profile in the central nervous system may explain some of the encephalopathic symptoms that often occur early in sepsis.

The experiment shows that the disturbance of protein metabolism with derangement of plasma and brain amino acids occurs early in sepsis. It supports the early correction of protein metabolism and plasma amino acid imbalance parallel to other therapeutic measures of sepsis. The use of a special amino acid solution with high levels of branched-chain amino acids has resulted in the normalization of the plasma amino acid pattern and an improvement of septic encephalopathy in one study (72). This is similar to the effect seen in the treatment of PSE; it supports the presence of a common mechanism of metabolic encephalopathies (further studies are necessary to clarify this).

Plasma and brain amino acids in uremia (VI)

The models of chronic and acute uremia gave rise to similar amino acid changes in plasma. Animals with chronic uremia had only a small deterioration of renal function with a moderate increase of BUN. These animals also showed minor amino acid changes in plasma as compared with animals with acute uremia. Plasma branched-chain amino acids, lysine, arginine, and serine decreased in both groups. The decrease of plasma branched-chain amino acids has been described in patients with renal failure (100, 101), and it may be a reflection of low protein intake and inappropriate hyperinsulinism (102). The low levels of plasma branched-chain amino acids in uremia were in the same range as seen after PCA. Plasma total tryptophan decreased in both uremic groups. This is reported by others in uremia (76) and in that study, an abnormally high proportion of the tryptophan was found in the free state. Plasma alanine levels were elevated in uremic rats, a finding also reported by others and thought to be due to an increased formation and release from skeletal muscle (103).

In brain, there were increases of histidine, phenylalanine, citrulline, and tyrosine. Similar findings are reported by others in CSF of patients and in experimental animals with uremia (76, 104). As the plasma levels of the neutral amino acids histidine, phenylalanine, and tyrosine were normal, their elevation in brain reflected a decreased competition for blood-brain transport among neutral amino acids by the decreased concentrations of branched-chain amino acids. Their elevation in brain seemed to be higher, though, than could be expected from the altered plasma competition, as shown in Figure 5 for phenylalanine.

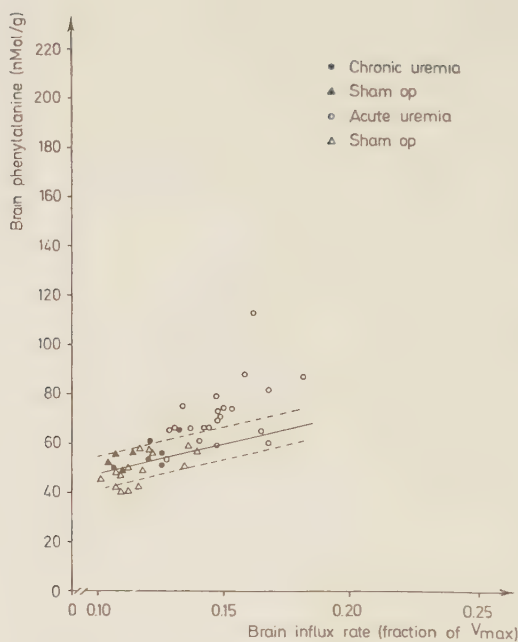


Figure 5. The relation between brain phenylalanine and brain influx rate (fraction of V_{max}) in uremic and sham-operated rats. The dashed lines are placed 2 S.D. above and below the control regression line.

This suggests that there may be an increased transport of neutral amino acids into brain in uremia.

The presence of an increased blood-brain neutral amino acid transport is further supported by the findings of increased brain uptake indices for tryptophan and tyrosine in uremic rats, but unchanged brain uptake index for lysine.

The altered plasma competition among neutral amino acids and increased blood-brain transport thus act synergistically to raise brain levels of neutral amino acids in uremia. Brain tryptophan was normal in uremic rats in this study and similar findings have been shown by others in experimental uremia (105). In humans with uremic encephalopathy, elevated CSF tryptophan levels have been reported (76). The increased plasma levels of free tryptophan and the decreased levels of competitors for entry to the brain reported by others (76, 105) and the finding of increased BUI for tryptophan in this study indicate that there is an increased transport of tryp-

tophan into brain in uremia. At the same time there seems to be an increased brain serotonin turnover in uremia with an accumulation of its metabolite 5-hydroxyindoleacetic acid (76), and this may explain the normal brain tryptophan levels.

The findings of increased brain neutral amino acid concentrations in uremia in this experiment are similar to those described by this group and others in PSE in experimental animals (57,58), although not to the same extent. In this condition, the high brain neutral amino acid levels are intimately involved in neurotransmitter changes and the etiology of PSE.

It was recently reported that patients with uremic encephalopathy also have disturbance of brain neurotransmitters. Increased levels of octopamine have been reported in plasma and CSF of patients with uremic encephalopathy (61, 75). Jellinger and Riederer (106) have shown in human post-mortem brains a decrease of dopamine and an increase of serotonin and 5-hydroxyindoleacetic acid in uremic encephalopathy. The encephalopathy of uremia thus seems to be accompanied by the same disturbances of cerebral neurotransmitters as encountered in PSE, and these may play an etiological role in the encephalopathy.

The encephalopathies of liver failure, sepsis, and uremia may be caused by a common underlying pathophysiology of increased blood-brain neutral amino acid transport and disturbance of cerebral neurotransmitters. All conditions are associated with severe derangement of protein and amino acid metabolism and the correction of this may benefit the encephalopathies, as has been shown with PSE (50).

SUMMARY

The present work deals with mechanisms underlying the encephalopathies seen in liver failure and portal-systemic shunting of blood, sepsis, and uremia. The symptomatology of these metabolic encephalopathies exhibits great similarities, suggesting a common basic pathophysiology.

The encephalopathy associated with hepatic dysfunction or portal-systemic shunting (PSE) has been studied most extensively, and most studies have concerned cerebral toxins. Presumably, toxins arising in the gut, normally metabolized by the liver, bypass the liver when blood is diverted around the liver parenchyma, or when hepatic function fails, and accumulate in the systemic circulation to poison the central nervous system. Ammonia has been the traditional villain. The proposed principal effect of ammonia has been its inhibition of cerebral energy metabolism, but its mechanism of action has not been clarified. More recently, interest was focused on the detoxification of ammonia by the brain by conversion of glutamic acid to glutamine. Elevation of glutamine in CSF and brain is a consistent finding in humans and experimental animals with PSE. The function of glutamine in brain is not clear; at least in animals, glutamine is transported out of brain after PCA. It has been proposed that ammonia toxicity is a function of ammonia detoxification with a decrease of brain glutamate and elevation of glutamine, and not a consequence of ammonia concentrations per se, but again the mechanism of action is not obvious.

The subtle derangement of cerebral dysfunction seen in PSE suggests that an altered neurotransmission may be taking place. Several investigators have established the presence of neurotransmitter derangements in PSE, with elevation of brain serotonin, depletion of norepinephrine and dopamine and an accumulation of the false neurotransmitters octopamine and phenylethanolamine. These changes may play an etiological role in PSE and this concept has been proposed as the primary underlying mechanism as opposed to the concept of cerebral toxins.

The neurotransmitter alterations have been shown to be caused by an accumulation in CNS of aromatic amino acids. This in turn is secondary to an imbalance of plasma amino acids in hepatic dysfunction with low levels of branched-chain amino acids and high levels of aromatic amino acids, resulting in an altered competition among neutral amino acids for blood-brain transport, but also to an increased transport activity of neutral amino acids at the blood-brain barrier.

The performed experiments lend support to a new mechanism whereby hyperammonemia and plasma amino acid imbalance act synergistically to raise brain aromatic amino acids and thereby affecting neurotransmission.

In rats with PCA, brain glutamine levels correlate better with brain neutral amino acids than plasma competitor functions. Taken together, plasma competitor functions and brain glutamine concentration predict brain neutral amino acids better than do either factor separately, suggesting that their effects on brain neutral amino acids are separate and additive. As glutamine is produced in the brain and as a neutral amino acid has been shown to compete with other neutral amino acids for transport at the blood-brain barrier, it is reasonable to think that as glutamine is transported out of the brain by the carrier of the neutral amino acid system, when the carrier reaches the outer portion of the blood-brain barrier, it then will carry a large neutral amino acid, presumably an aromatic amino acid present in abundance in the plasma, into the brain, increasing its concentration.

Hyperammonemia influences the brain neutral amino acid concentrations via brain glutamine synthesis. It also lowers plasma branched-chain amino acids, whereby altering plasma competition among neutral amino acids and facilitating the entry of aromatic amino acids into brain.

The penetration of neutral amino acids into brain is thus influenced by hyperammonemia, brain glutamine synthesis, and competition in plasma among neutral amino acids. All these factors seem to be independent of gut bacteria, as they are indistinguishably present in germ-free rats with PCA.

This concept implies that therapeutic benefit may be obtained in PSE from a variety of methods, which either lower ammonia (lactulose, antibiotics, protein restriction), correct plasma amino acid imbalance (infusion of branched-chain amino acids), or act as neuromodulators (L-dopa, bromocriptine).

Rats with abdominal sepsis and uremia exhibit alterations of plasma and brain neutral amino acids similar to those seen after PCA, although to a lesser degree. There also seems to be an increased transport of neutral amino acids across the blood-brain barrier in both conditions. The elevated brain neutral amino acids may affect cerebral neurotransmission and thus play an etiological role in the encephalopathies seen in these conditions.

Liver failure, sepsis, and uremia are often associated with disturbances of protein and amino acid metabolism. Plasma amino acid imbalance may affect the cerebral function in all three conditions, as they seem to share a common derangement of the blood-brain barrier. The early and vigorous treatment of protein and amino acid imbalance thus seems to be of great importance for the correction of the encephalopathies of the diseases.

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REFERENCES

1. Conn H O, Lieberthal M M: The hepatic coma syndromes and lactulose. The Williams & Wilkins Co, Baltimore, 1979
2. Hahn M, Massen O, Nencki M, Pawlow J: Die Eck'sche Fistel zwischen der unteren Hohlvene und der Pfortader und ihre Folgen für den Organismus. Arch exp Pathol Pharmacol 32:161-210, 1893
3. Schwartz R, Phillips G B, Gabuzda G J Jr, Davidson C S: Blood ammonia and electrolytes in hepatic coma. J Lab Clin Med 42:499-508, 1953
4. Stahl J: Studies of the blood ammonia in liver disease. Its diagnostic, prognostic and therapeutic significance. Ann Int Med 58:1-24, 1963
5. Summerskill W H J, Wolfe S J, Davidson C S: The metabolism of ammonia and α -keto acids in liver disease and hepatic coma. J Clin Invest 36:361-372, 1957
6. Mutchnick M G, Lerner E, Conn H O: Portal-systemic encephalopathy and portacaval anastomosis: a prospective, controlled investigation. Gastroenterology 66:1005-1019, 1974
7. Conn H O, Leevy C M, Vlahcevic Z R, Rodgers J B, Maddrey W C, Seeff L, Levy L L: Comparison of lactulose and neomycin in the treatment of chronic portal-systemic encephalopathy. Gastroenterology 72:573-583, 1977
8. Folin O, Denis W: Protein metabolism from the standpoint of blood and tissue analysis. II. The origin and significance of the ammonia in the portal blood. J Biol Chem 11:161-167, 1912
9. Phillips G B, Schwartz R, Gabuzda G J Jr, Davidson C S: The syndrome of impending hepatic coma in patients with cirrhosis of the liver given certain nitrogenous substances. N Engl J Med 247:239-246, 1952
10. Nance F C, Batson R C, Kline D G: Ammonia production in germ-free Eck fistula dogs. Surgery 70:169-174, 1971
11. Weber F L, Veach G L: The importance of the small intestine in gut ammonium production in the fasting dog. Gastroenterology 77:235-240, 1979
12. Richterich R W, Goldstein L: Distribution of glutamine metabolizing enzymes and production of urinary ammonia in mammalian kidney. Am J Physiol 195:316-320, 1958
13. Alles S I, Conn H O: Observations on the effect of exercise on blood ammonia concentration in man. Yale Journal of Biological Medicine 33:133-144, 1960

14. Duda G D, Hendler P: Kinetics of ammonia metabolism in vivo. *J Biol Chem* 232:303-314, 1958
15. Schenker S, Breen K J, Hoyumpa A M Jr: Hepatic encephalopathy: current status. *Gastroenterology* 66:121-151, 1974
16. Bessman S P: Ammonia and Coma. *Chemical Pathology of the Nervous System*. J Folch-Pi (ed.). Pergamon Press, N Y, 1961, pp 370-376
17. Shorey J, McCandless D W, Schenker S: Cerebral α -ketoglutarate in ammonia intoxication. *Gastroenterology* 53:706-711, 1967
18. Berl S: Cerebral amino metabolism in hepatic coma. *Exp Biol Med* 4:71-84, 1971
19. Hindfelt B, Siesjö B K: Cerebral effects of acute ammonia intoxication. II. The effect upon energy metabolism. *Scand J clin Lab Invest* 28:365-374, 1971
20. Hindfelt B, Plum F, Duffy T E: Effect of acute ammonia intoxication on cerebral metabolism in rats with portacaval shunts. *J Clin Invest* 59:386-396, 1977
21. Holmin T, Siesjö B K: The effect of porta-caval anastomosis upon the energy state and upon acid-base parameters of the rat brain. *J Neurochem* 22:403-412, 1974
22. Hawkins R A, Miller A L, Nielsen R C, Veech R L: The acute action of ammonia on rat brain metabolism in vivo. *Biochem J* 134:1001-1008, 1973
23. Johnson J L: Glutamic acid as a synaptic transmitter in the nervous system. A review. *Brain Res* 37:1-19, 1972
24. Gjedde A, Lockwood A H, Duffy T E, Plum F: Cerebral blood flow and metabolism in chronically hyperammonemic rats: effect of an acute ammonia challenge. *Ann Neurol* 3:325-330, 1978
25. Hourani B T, Hamlin E M, Reynolds T B: Cerebrospinal fluid glutamine as a measure of hepatic encephalopathy. *Arch Intern Med* 127:1033-1036, 1971
26. Flock E V, Block M A, Grindlay J H, Mann F C, Bollman J L: Changes in free amino acids of brain and muscle after total hepatectomy. *J Biol Chem* 200:529-536, 1953
27. Williams A H, Kyu M H, Fenton J C B, Cavanagh J B: The glutamate and glutamine content of rat brain after portocaval anastomosis. *J Neurochem* 19:1073-1077, 1972
28. Baldessarini R J, Yorke C: Uptake and release of possible false neurotransmitter amino acids by rat brain tissue. *J Neurochem* 23:839-848, 1974

29. Vergara F, Plum F, Duffy T E: α -Ketoglutamamate: increased concentrations in the cerebrospinal fluid of patients in hepatic coma. *Science* 183:81-83, 1974
30. Warren K S, Schenker S: Effect of an inhibitor of glutamine synthesis (methionine sulfoximine) on ammonia toxicity and metabolism. *J Lab Clin Med* 64:442-449, 1964
31. Hindfelt B, Plum F: L-methionine DL-sulphoximine and acute ammonia toxicity. *J Pharm Pharmacol* 27:456-458, 1975
32. Chen S, Mahadevan V, Zieve L: Volatile fatty acids in the breath of patients with cirrhosis of the liver. *J Lab Clin Med* 75:622-627, 1970
33. Rabinowitz J L, Staeffen J, Blanquet P, Vincent J D, Terme R, Series C, Myerson RM: Sources of serum ^{14}C -octanoate in cirrhosis of the liver and hepatic encephalopathy. *J Lab Clin Med* 91:223-227, 1978
34. Muto Y: Clinical study on the relationship of short-chain fatty acids and hepatic encephalopathy. *Jpn J Gastroenterology* 63:19-32, 1966
35. Zieve L: Hepatic encephalopathy: Summary of present knowledge with an elaboration on recent developments. *In* *Progress in Liver Disease* (eds.) Popper H, Schaffner F, vol 6 pp 327-341, Grune & Stratton, N Y 1979
36. Zieve L, Doizaki W M, Zieve F J: Synergism between mercaptans and ammonia or fatty acids in the production of coma: a possible role for mercaptans in the pathogenesis of hepatic coma. *J Lab Clin Med* 83: 16-28, 1974
37. McClain C J, Zieve L, Doizaki W M, Gilberstadt S, Onstad G R: Blood methanethiol in alcoholic liver disease with and without hepatic encephalopathy. *Gut* 21:318-323, 1980
38. McMenamy R H, Vang J, Drapanas T: Amino acids and α -keto acid concentrations in plasma and blood of the liverless dog. *Am J Physiol* 209(5):1046-1052, 1965
39. Mans A M, Saunders S J, Kirsch R E, Biebuyck J F: Correlation of plasma and brain amino acid and putative neurotransmitter alterations during acute hepatic coma in the rat. *J Neurochem* 32:285-292, 1979
40. Zanchin G, Rigotti P, Dussini N, Vassanelli P, Battistin L: Cerebral amino acid levels and uptake in rats after portocaval anastomosis: II. Regional studies in vivo. *J Neurosci Res* 4:301-310, 1979
41. Smith A R, Rossi-Fanelli F, Ziparo V, James J H, Perelle B A, Fischer J E: Alterations in plasma and CSF amino acids, amines and metabolites in hepatic coma. *Ann Surg* 187:343-350, 1978

42. Smith A R, Rossi-Fanelli F, Freund H, Fischer J E: Sulfur-containing amino acids in experimental hepatic coma in the dog and the monkey. *Surgery* 85:677-683, 1979
43. Rosen H M, Yoshimura N, Hodgman J M, Fischer J E: Plasma amino acid patterns in hepatic encephalopathy of differing etiology. *Gastroenterology* 72:483-487, 1977
44. Chase R A, Davies M, Trewby P N, Silk D B A, Williams R: Plasma amino acid profiles in patients with fulminant hepatic failure treated by repeated polyacrylonitrile membrane hemodialysis. *Gastroenterology* 75:1033-1040, 1978
45. Record C O, Buxton B, Chase R A, Curzon G, Murray-Lyon I M, Williams R: Plasma and brain amino acids in fulminant hepatic failure and their relationship to hepatic encephalopathy. *Eur J Clin Invest* 6:387-394, 1976
46. Cascino A, Cangiano C, Calcaterra V, Rossi-Fanelli F, Capocaccia L: Plasma amino acids imbalance in patients with liver disease. *Am J Dig Dis* 23:591-598, 1978
47. Soeters P B, Fischer J E: Insulin, glucagon, aminoacid imbalance, and hepatic encephalopathy. *Lancet* ii:880-882, 1976
48. Sherwin R, Joshi P, Hendler R, Felig P, Conn H O: Hyperglucagonemia in Laennec's cirrhosis. The role of portal-systemic shunting. *N Engl J Med* 290:239-242, 1974
49. Munro H N, Fernstrom J D, Wurtman R J: Insulin, plasma aminoacid imbalance, and hepatic coma. *Lancet* i:722-724, 1975
50. Fischer J E, Rosen H M, Ebeid A M, James J H, Keane J M, Soeters P B: The effect of normalization of plasma amino acids on hepatic encephalopathy in man. *Surgery* 80:77-91, 1976
51. Fernstrom J D, Faller D V: Neutral amino acids in the brain: Changes in response to food ingestion. *J Neurochem* 30:1531-1538, 1978
52. Pardridge W M: Kinetics of competitive inhibition of neutral amino acid transport across the blood-brain barrier. *J Neurochem* 28:103-108, 1977
53. Sato Y, Eriksson S, Hagenfeldt L, Wahren J: Influence of branched-chain amino acid infusion on arterial concentrations and brain exchange of amino acids in patients with hepatic cirrhosis. *Clinical Physiology*, 1981, in press
54. Ono J, Hutson D G, Dombro R S, Levi J U, Livingstone A, Zeppa R: Tryptophan and hepatic coma. *Gastroenterology* 74:196-200, 1978

55. Bloxam D L, Curzon G: A study of proposed determinants of brain tryptophan concentration in rats after portocaval anastomosis or sham operation. *J Neurochem* 31:1255-1263, 1978
56. James J H, Escourrou J, Fischer J E: Blood-brain neutral amino acid transport activity is increased after portacaval anastomosis. *Science* 200:1395-1397, 1978
57. James J H, Hodgman J M, Funovics J M, Yoshimura N, Fischer J E: Brain tryptophan, plasma free tryptophan and distribution of plasma neutral amino acids. *Metabolism* 25:471-476, 1976
58. Curzon G, Kantamaneni B D, Fernando J C, Woods M S, Cavanagh J B: Effects of chronic porto-caval anastomosis on brain tryptophan, tyrosine and 5-hydroxytryptamine. *J Neurochem* 24:1065-1070, 1975
59. Freund H, Krause R, Rossi-Fanelli F, Smith A R, Fischer J E: Amino acid induced coma in normal animals; prevention by branched-chain amino acids. *Gastroenterology* 74:1167, 1978
60. Fischer J E, Baldessarini R J: False neurotransmitters and hepatic failure. *Lancet* ii:75-80, 1971
61. Lam K C, Tall A R, Goldstein G B, Mistilis S P: Role of a false neurotransmitter, octopamine, in the pathogenesis of hepatic and renal encephalopathy. *Scand J Gastroent* 8:465-472, 1973
62. Cangiano C, Rossi-Fanelli F, Bozzi A, Calcaterra V, Cascino A, Capocaccia L: Plasma phenylethanolamine in hepatic encephalopathy. *Eur J Clin Invest* 8:183-184, 1978
63. Jellinger K, Riederer P, Kleinberger G, Wuketich St, Kothbauer P: Brain monoamines in human hepatic encephalopathy. *Acta neuropathol (Berl.)* 43:63-68, 1978
64. Dodsworth J M, James J H, Cummings M C, Fischer J E: Depletion of brain norepinephrine in acute hepatic coma. *Surgery* 75:811-820, 1974
65. Baldessarini R J, Fischer J E: Trace amines and alternative neurotransmitters in the central nervous system. *Biochem Pharm* 27:621-626, 1978
66. Young S N, Lal S: CNS tryptamine metabolism in hepatic coma. *J Neural Transmission* 47:153-161, 1980
67. Faraj B A, Camp V M, Ansley J D, Scott J, Ali F, Malveaux E J: Evidence for central hypertyraminemia in hepatic encephalopathy. *J Clin Invest* 67:395-402, 1981
68. Fischer J E, Funovics J M, Falcao H A, Wesdorp R I C: L-Dopa in hepatic coma. *Ann Surg* 183:386-391, 1976

69. Morgan M Y, Jakobovits A W, James I M, Sherlock S: Successful use of bromocriptine in the treatment of chronic hepatic encephalopathy. *Gastroenterology* 78:663-670, 1980
70. Striebel J P, Holm E, Lutz H, Storz L W: Parenteral nutrition and coma therapy with amino acids in hepatic failure. *JPEN* 3:240-246, 1979
71. Capocaccia L, Calcaterra V, Cangiano C, Cascino A, Fiaccadori F, Gentile S, Ghinelli F, Pelosi G, Riggio O, Rossi-Fanelli F, Sacchini D, Giunchi G: Therapeutic effect of branched chain amino acids in hepatic encephalopathy: a preliminary study. In *Serono Symposium No. 34, "Medical and Surgical Problems of Portal Hypertension"*, M J Orloff, S Stipa (eds.), Academic Press, London and New York, 1980, pp 239-249
72. Freund H R, Ryan J A Jr, Fischer J E: Amino acid derangements in patients with sepsis: treatment with branched chain amino acid rich infusions. *Ann Surg* 188:423-430, 1978
73. Freund H, Atamian S, Holroyde J, Fischer J E: Plasma amino acids as predictors of the severity and outcome of sepsis. *Ann Surg* 190:571-576, 1979
74. Raskin N H, Fishman R A: Neurologic disorders in renal failure. *N Engl J Med* 294:143-148, 1976
75. Kinniburgh D W, Boyd N D: Determination of plasma octopamine and its level in renal disease. *Clin Biochem* 12:27-32, 1979
76. Sullivan P A, Murnaghan D, Callaghan N, Kantamaneni B D, Curzon G: Cerebral transmitter precursors and metabolites in advanced renal disease. *J Neurol Neurosurg Psych* 41:581-588, 1978
77. Funovics J M, Cummings M G, Shuman L, James J H, Fischer J E: An improved nonsuture method for portacaval anastomosis in the rat. *Surgery* 77:661-664, 1975
78. Wichterman K A, Chaudry, I H, Baue A E: Studies of peripheral glucose uptake during sepsis. *Arch Surg* 114:740-745, 1979
79. Russell J E, Avioli L V: Effect of experimental chronic renal insufficiency on bone mineral and collagen maturation. *J Clin Invest* 51: 3072-3079, 1972
80. Iaina A, Solomon S, Eliahou H E: Reduction in severity of acute renal failure in rats by beta-adrenergic blockade. *Lancet* ii:157-159, 1975
81. Bruce A W, Leiendecker C M, Freier E F: Two-point determination of plasma ammonia with the centrifugal analyzer. *Clin Chem* 24:782-787, 1978

82. Oldendorf W H: Brain uptake of radiolabeled amino acids, amines, and hexoses after arterial injection. *Am J Physiol* 221(6):1629-1639, 1971
83. Oldendorf W H, Szabo J: Amino acid assignment to one of three blood-brain barrier amino acid carriers. *Am J Physiol* 230(1):94-98, 1976
84. Pardridge W M, Oldendorf W H: Kinetic analysis of blood-brain barrier transport of amino acids. *Biochim Biophys Acta* 401:128-136, 1975
85. Hjelle J T, Baird-Lambert J, Cardinale G, Spector S, Udenfriend S: Isolated microvessels: the blood-brain barrier in vitro. *Proc Natl Acad Sci USA* 75:4544-4548, 1978
86. Lowry O H, Rosebrough N J, Farr L A, Randall R J: Protein measurements with the folin phenol reagent. *J Biol Chem* 193:265-275, 1951
87. Bruning J L, Kintz D L: Computational calculations of statistics. II ed. Scott, Foresman Co., Glenview, Ill., 1977, pp 187 ff
88. Toth J, Lajtha A: Rates of exchange of free amino acids between plasma and brain in mice. *Neurochem Res* 2:149-160, 1977
89. Cardelli-Cangiano P, Cangiano C, James J H, Jeppsson B, Brenner W, Fischer J E: Uptake of amino acids by brain microvessels isolated from rats after portacaval anastomosis. *J Neurochem* 36:627-638, 1981
90. Marco J, Diego J, Villanueva M L, Díaz-Fierros M, Valverde I, Segovia J M: Elevated plasma glucagon levels in cirrhosis of the liver. *N Engl J Med* 289:1107-1111, 1973
91. Strombeck D R, Rogers Q, Stern J S: Effects of intravenous ammonia infusion on plasma levels of amino acids, glucagon and insulin in dogs. *Gastroenterology* 74:1165, 1978
92. Creutzfeldt W, Frerichs H, Sickinger K: Liver diseases and diabetes mellitus. In *Progress in Liver Diseases*, vol 3, (eds.) Popper H, Schaffner F, 371-407, London, Heineman, 1970
93. Schlienger J L, Imler M: Effect of hyperammonemia on insulin-mediated glucose uptake in rats. *Metabolism* 27:175-183, 1978
94. Felig P: Amino acid metabolism in man. *Ann Rev Biochem* 44:933-955, 1975
95. Wostmann B S: Nutrition and metabolism of the germ-free mammal. *World Review of Nutrition and Dietetics* 22:40-92, 1975
96. Wichterman K A, Baue A E, Chaudry I H: Sepsis and septic shock - a review of laboratory models and a proposal. *J Surg Res* 29:189-201, 1980

97. Clowes G H A Jr, O'Donnell T F, Blackburn G L, Maki T N: Energy metabolism and proteolysis in traumatized and septic man. *Surg Clin North Am* 56:1169-1185, 1976
98. Wannemacher R W Jr, Powanda M C, Dinterman R E: Amino acid flux and protein synthesis after exposure of rats to either diplococcus pneumoniae or salmonella typhimurium. *Infect Immun* 10:60-65, 1974
99. Wolf L I, Groves A C, Duff J H: Amino acid metabolism in dogs with E. coli bacteremic shock. *Surgery* 85:212-218, 1979
100. Fürst P, Alvesstrand A, Bergström J: Effects of nutrition and catabolic stress on intracellular amino acid pools in uremia. *Am J Clin Nutr* 33:1387-1395, 1980
101. Kopple J D, Jones M, Fukuda S, Swendseid M E: Amino acid and protein metabolism in renal failure. *Am J Clin Nutr* 31:1532-1540, 1978
102. Rubenfeld S, Garber A J: Abnormal carbohydrate metabolism in chronic renal failure. The potential role of accelerated glucose production, increased gluconeogenesis, and impaired glucose disposal. *J Clin Invest* 62:20-28, 1978
103. Garber A J: The regulation of skeletal muscle alanine and glutamine formation and release in experimental chronic uremia in the rat. *J Clin Invest* 62:633-641, 1978
104. Müting D, Dishuk B D: Free amino acids in serum, cerebrospinal fluid, and urine in renal disease with and without uremia. *Proc Soc exp Biol (N Y)* 126:754-758, 1967
105. Siassi F, Wang M, Kopple J D, Swendseid M E: Brain serotonin turnover in chronically uremic rats. *Am J Physiol* 232(5):E526-E528, 1977
106. Jellinger K, Riederer P: Brain monoamines in metabolic (endotoxic) coma. A preliminary biochemical study in human postmortem material. *J Neural Transmission* 41:275-286, 1977

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